

A STUDY OF SPIROCHETES IN CHICKENS

WITH SPECIAL REFERENCE TO THOSE OF THE
INTESTINAL TRACT

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Introduction.

Spirochetes in birds were first described by Sacharoff in 1890 and since then the work in this field has dealt for the most part with those spirochetes which are pathogenic to birds, especially *Spirochaeta gallinarum*, the etiological agent in fowl spirochaetosis.

The present investigation was undertaken to determine to what extent spirochetes might be found in birds, particularly chickens, even though fowl spirochaetosis is not prevalent in Maryland and vicinity.

Historical.

Spiral or undulating microorganisms have been described since the early days of microscopy. Because of almost complete ignorance of this field of biology and of the primitive types of magnifying instruments in use at that time the recorded observations are, in the light of our present knowledge, confused and inaccurate; and the organisms described are difficult if not impossible to identify with any certainty from the data available.

Gleichen and Joblot, mentioned by Ehrenberg, used the term "Zit-terthierchen" for microorganisms impossible of identification by

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Ehrenberg. Mueller worked over Linnaeus' attempt to classify the material described by Leeuwenhoeck, and by means of an improved microscope which magnified about 300 diameters he doubtless observed more bacteria than the large bacteria which Leeuwenhoeck, working with a magnification of perhaps 160 diameters, is believed to have seen. In his classification of microorganisms (1786) Mueller lists "Monas" as spherical or ovoid and "Vibrio" as longer and in most species spiral (36). Mueller seems to have been the first to suggest a separate classification for long bending or twisting microorganisms under the term "Vermes." From a review of the literature available, the reports of Ehrenberg appear to embody the earliest form of our present conceptions regarding spiral microorganisms. In his book "Die Infusionsthierchen" of 1838 he refers to his reports before the Berlin Academy in 1830 and 1833 and we find what may be presumed to be the conceptions of that period when more accurate microscopic observations were being made and the foundations of present day ideas of classification were being laid. Many of the terms now applied to spiral organisms have come down from that time. One deduces from Ehrenberg's statements that he groups all motile microorganisms (including spiral or undulating forms) under "Monads" and because of their motility considers them all to be animals. Doubtless, protozoa are included with his "Monads" (11). Of Monads he says the following: "All small bodies with independent motility revealed in water by the microscope at such relatively high magnifications that delicate external organs would be recognizable, which carry no feet, hairs, bristles or other external appendages and which also do not seem to be surrounded or armoured by any special mucoid, cuticular, or hard covering; among which furthermore several digestive vacuoles within the body are distinctly visible or very probably present—but in which a connecting alimentary canal cannot be clearly demonstrated; which never appear as chains consisting of links but at the most occasionally are doubled by simple constriction (division—Selbsttheilung) or divide at right angles into four or mulberry-like forms and whose spherical, oval, or elongated bodies show no voluntary change in form, either at rest or when swimming, such bodies belong to the family of Monads. The twenty-five varieties of the division of monads may be grouped: 1, Ball monads or spherical or egg shape of which there are 17, and 2, Rod monads with elongated form, more than twice as long as they are thick—of which there are 8.*

* "Alle selbstbewegten Körperchen welche das Microscop im Wasser zeigt, die bei verhältnismässiger so starker Vergrösserung, dass äussere zarte Organe erkennbar

He noted the apparent contradiction that "Bacterium" while motile, and therefore placed in his group of animal Monads, has characteristics in common with non-motile bacteria of similar rod shapes or even with those of spherical or oval form. He says that the actual reason why these little forms are here regarded as animals lies on the one hand in the very energetic swimming serpentine and obviously independent motion which these forms distinctly possess and which has established a place among the animals for them from the beginning. On the other hand he says that he recognized in that most distinct form and division of "Bacterium" an organ of motility in the shape of a simple whirling proboscis which is decisive evidence of animal nature and which presents an analogy for the other forms supporting the great likelihood of a similar structure in those cases.* Under the "Monads" he places the "Zitterthierchen" (trembling animalcules) or "Vibrionia" or "Vibrionides," the motile thread-like forms. These he describes in the following manner: "In the family of 'Zitterthierchen' belong all those small thread-like bodies which have independent motility and are jointed and to which appertain all the characters of the monads so far as these may be ascertained; which are actually or probably polygastrie, without alimentary tract, without covering or external appendages and without rigid bodies and whose thread-like form results from incomplete transverse division. Or:—'Zitterthierchen are monads which form motile jointed chains by incomplete transverse division.

werden konnten, keine Füße, Haare, Borsten, oder andere äussere Anhänge führen, die auch nicht von irgend einer besondern gallertigen, hautigen, oder harten Hülle umgeben und gepanzert erscheinen; bei denen ferner zwar sich eine Mehrzahl von blasenartigen Speisebehältern im innern Körper erkennen lässt, oder sehr wahrscheinlich wird, aber kein dieser verbindender Speisekanal zur Klarheit gebracht werden kann, die nie kettenartig gegliedert erscheinen, sondern nur höchstens zuweilen durch einfache Einschnürung (Selbsttheilung) doppelt, oder durch kreuzweise Einschnürung viertheilig oder brombeerartig werden und deren kugliger, eiförmiger, oder länglicher Körper beim Ruhen und Schwimmen keine willkürliche Formveränderung zeigt, solche Körper gehören zur Familie der Monaden. Die 25 Arten der Gattung Monas lassen sich nach ihrer Gestalt in 2 Gruppen übersichtlich machen: 1. als Kugelmonaden von ganz runder oder eiertiger Form, deren sind 17. und 2. -als Stabmonaden mit länglicher, mehr als doppelt so langer als dicker Form, solcher sind 8."

* "Der Grund, warum nun überhaupt diese Körperchen hier als Thiere angesehen werden liegt einerseits in der sehr kräftigen, schwemmenden, schlängelnden, offenbar willkürlichen Bewegung, welche diese Formen deutlich besitzen und die von jeher ihnen eine Stelle bei den Thieren gesichert hat, allein ich habe auch bei der stärksten Art und Gattung 'Bacterium' ein Bewegungsorgan als einfachen wirbelnden Rüssel erkannt, welches über die Thierheit derselben schon völlig entscheidet, und welches auch für die übrigen eine Analogie darstellt, die die grosse Wahrscheinlichkeit gleicher Bildung befestigt."

The number of species which constitute this family number 14. They are divided into 5 groups. *Vibrio* with 6 kinds, *Bacterium* and *Spirillum* each with 3 kinds, *Spirochaeta* and *Spirodiscus* each with 1 kind." *

"The motion of the true vibrios is serpentine in that the straight jointed chain bends like a snake and again straightens out when at rest. The reason for this appears to lie in the greater constriction and greater separation of the unit 'animals' so that these are able to displace themselves by sliding against each other. In the case of *Bakterium* the constriction is less, therefore no serpentine motion is possible, merely 'straight swimming,' " is the statement of Ehrenberg.†

The family of spirilla was first reported in 1830 (12) before the Berlin Academy and described as a family of such "animalcules" of the *Vibrionides* which through incomplete division (slanting) produce spiral-shaped and rigid chains of cylindrical form or cylindrical screw forms.‡

It is in comparison and contrast with these two pictures of "vibrio" and "spirillum" that he describes his "*Spirochaeta plicatilis*." This form was placed among the "Zitterthierchen" or "Vibrionides" or "Vibrionia" as one of the five types in this class, the members as listed by him being, "*Vibrio*," "*Bacterium*," "*Spirillum*," "*Spirochaeta*" and "*Spirodiscus*." *Spirodiscus* is a form which appears to be outside of the group of organisms in which we are here interested.

Ehrenberg, in 1833 (13) described "the family of spirochetes as one form of the vibration animalcules, 'vibrionen,' which is distinguished

* "Zitterthierchen-Vibrionia-Vibrionides—Es gehören zur Familie der Zitterthierchen alle fadenartigen Körperchen, welche selbst bewegt und gegliedert sind und die all Charactere der Monadenfamilie an sich tragen, so weit diese erreichbar sind; die wirklich oder wahrscheinlich polygastrisch, darmlos, panzerlos, ohne äussere Anhänge und unveränderlicher Körperform sind, und deren fadenartige Gestalt durch unvollkommene queere Selbsttheilung entsteht. Oder: Zitterthierchen sind Monadinen, welche durch queere unvollkommene Selbsttheilung bewegte Gliederfaden bilden. Sie sind in 5 Gattungen vertheilt: *Vibrio* mit 6 Arten, *Bacterium* und *Spirillum* jede mit 3 Arten, *Spirochaeta* und *Spirodiscus* jede mit 1 Art."

† "Die Bewegung der wahren Vibrionen ist eine schlängelnde, so dass der gerade Gliederfaden sich schlangenartig krümmt und in die Ruhe wieder streckt. Der Grund davon scheint in einer stärkeren Einschnürung und grösseren Isolirung der Einzelthiere zu liegen, so dass sich diese an einander verschieben können. Beim *Bakterium* ist die Einschnürung daher kein Schlängeln möglich, nur ein gerades Schwimmen."

‡ "Walzenspiralen sind solche Thierchen der Vibrionen-Familie, welche aus unvollkommener (schiefer?) Selbsttheilung hervorgegangene spiralförmige und unbiegsame Ketten von cylindrischer Form, oder Schraubencylinder bilden. Die Gattung wurde zuerst im Jahre 1830 in den Abhandlungen der Berliner Akademie angezeigt."

from the rest by its incomplete (slanting?) division which results in a coiled flexible chain form, or a thread-like screw form,—an elongated flexible spirillum. The family was first proposed in the Proceedings of the Berlin Academy in 1833. It is based on a single animal form which is indeed very distinctive in that it combined the rigidity of the cylindrical spirals (spirillum) with the flexibility of the vibrios. It was an animalcule of great activity, resembling the spiral fibrils of plants, which hurled itself hither and yon without losing its spiral form, bent in serpentine fashion and exhibited much energy. Nothing could be ascertained of the organization of this extraordinarily delicate spiral thread except that it consisted of small closely crowded units which resembled those of vibrios and which might therefore be single organisms whose structural detail is concealed from us because of their minute size and the limitations of our present optical apparatus. Observations were not made except in Berlin".*

Spirochaeta plicatilis is an organism which "stands" between the vibrios and spirilla and was observed by Ehrenberg (14) on April 2nd 1832 in water which had stood over winter in his residence in Berlin. It was a closely coiled organism, very delicate, colorless, stretched out like a long thread, and without losing its spiral form bent vigorously like an earthworm in the most varied shapes, and swam about in serpentine manner like a eel. The organism was about 70 times as long as wide, having a length of 55 to 84 microns and a width of 1 micron. On drying out the details became visible.†

* "Die Gattung der Schlingenthierchen ist eine Form der Zitterthierchen, Vibrionen, welche sich von den übrigen durch eine aus unvollkommener (shiefer?) Selbsttheilung hervorgegangenen gewundenen, aber dabei biegsamen Kettenform oder fadenartigen Schraubenform unterscheidet. (Ein verlängertes biegsames Spirillum). Die erste Aufstellung dieser Gattung geschah in den Abhandlungen der Berliner Akademie 1833, s. 313. Sie gründete sich auf eine einzelne Thierform welche freilich sehr ausgezeichnet war indem sie die starre Natur der Walzenspiralen (Spirillum) mit der Biegsamkeit der Vibrionen vereinigte. Es war ein Thierchen von grosser Lebendigkeit, welches einer spiralfaser, des Pflanzengewebes glich, die, ohne ihr Spiralforn zu verlieren, sich hin und her schleuderte, schlingenartig umbog und viel Energie erkennen liess.

"Von Organisation war dem ausserordentlich zartem Spiralfaden nichts weiter zu ermitteln, als dass es aus dicht an einander gedrängten Gliedern bestand, welche den Vibrionen-Gliedern glichen, und die daher Einzelthiere seien mögen, deren Organisation-Detail unserer jetzigen Sehkraft, seiner Feinheit halber, verschlossen ist. Ausser bei Berlin ist es nicht beobachtet."

† "Dieses zwischen Vibron und Spirillum stehende Thierchen fand sich am 2ten April 1832 im überwinterten Wasser in meiner Wohnung zu Berlin. Es war eng schraubenartig gewunden, sehr zart und farblos, dabei fadenartig gestreckt und bog sich ohne seine Schraubenform zu verlieren, wie ein Regenwurm, kräftig in die

In attempting to summarize Ehrenberg's observations and explanations of spiral forms one might perhaps assume the following: (1) In the "vibrios" the individual units are loosely joined in a long thread, and the joints which are formed by almost complete transverse division allow the units to slide against each other at those points and thus form undulations of the thread while in motion. (2) In the "spirillum" a similar succession of units is divided from each other by transverse (or perhaps slanting) incomplete division. He emphasizes the rigidity of the spiral thread characteristic of this class, but makes no definite statement that this rigidity is due to rigid joints, perhaps less completely divided. (3) In the "spirochete" finally he postulates also a succession of units forming a spiral thread, the units again being partially divided from each other by transverse (or perhaps slanting) incomplete division. He considers that it "stands between" the "vibrio" and "spirillum" and emphasizes its characteristic extreme flexibility. No definite statement is found however, regarding the nature of the hypothecated joints between the units in the chain to account for their flexibility. It may be remarked that the "slanting" division is suggested for spirillum and spirochete only, but again no definite statement is found relating this suggestion to the constant spiral form present in these two forms and not found in "vibrio". These terms have been used in various senses since then.

In 1873 (44) Obermeier reported a spirochete in relapsing fever in Eastern Europe. It is described as a rather stout organism, the spiral threads having a length of 7 to 9 microns, a diameter of 1 micron, and 4 to 10 undulations. Multiplication is by transverse division and motility is active and cork-screw-like with lateral oscillations. It is known as *Spironema recurrentis*, *Spirochaeta recurrentis*, and *Spirochaeta Obermeieri*.

Schaudinn and Hoffmann in 1905 (51) described a spirochete present in syphilis which was named *Spirochaeta pallida*, but later renamed and is now known as *Treponema pallidum*. This organism is from 4 to 10 microns long, up to 0.5 micron in diameter, and has from 8 to 12 spirals. It is more delicate than the organism of relapsing fever. A statement was made by Schaudinn in 1904 (52) that he was of the opinion that certain spirochetes constitute a phase in the life cycle of trypanosomes. It is not uncommon to employ the term "spirilla"

verschiedensten Gestalten, schwamm auch sich schlängelnd wie ein Aal. Die Dicke des Fadens lag bis 70 mal in seiner Länge. Länge der Fäden (Monadenstöcke) 1/18 bis 1/12 Linie. Dicke der Fäden,—1/1000 Linie. Beim Antrocknen wurde die Gliederung messbar."

and "spirochetes" interchangeably and this has been a source of much confusion, especially in reviewing the older literature. According to the old school it mattered not whether a spiral organism had one or two polar flagella or a tuft of flagella. Schaudinn on the other hand maintained that the distinction between spirilla and spirochetes was of considerable significance because he believed that one was of plant while the other was of animal origin. This view was rather revolutionary but was based upon his observations on a protozoan, *Leukocytozoan ziemanni*, found in the blood of the little owl (*Athene noctua*), and regarded by Schaudinn as a trypanosome, which was said to undergo a spirochetal stage while in the intermediate host (*Culex pipiens*). Although his work was questioned, it nevertheless had a far-reaching effect upon the development of our knowledge of spirochetes. The name *Spirochaeta pallida* was given to the newly discovered organism in syphilis in 1905 because of its resemblance to the spirochetes in general (53), but within a year Schaudinn noted certain features which were considered sufficiently distinctive to classify it apart from the usual spirochetes and he therefore used the name *Treponema* (54). Vuillemin in 1905 proposed the name *Spironema* for the same organism (62). Later other new generic names were created by the systematists as, *Microspironema* (57), *Borrelia* (58), *Spiroschaudinina* (48), and *Spirosoma* (55). These however, are not widely used today.

Whether spirochetes are bacteria or protozoa had been considered by some as unimportant, but to Swellengrebel it seemed a matter of considerable interest and in 1912 (59) he reported comparisons by morphological, chemical, and pathological methods. He stated at that time that morphologically the spirochetes have the following in common with bacteria: the inner structure, the definite (not rigid) membrane, the periblast appendages, terminal flagella, transverse division, and degeneration (granular destruction). They differ chiefly in that they have greater flexibility, in the fibrillar structure of the cell wall, and in their supposed ability to divide longitudinally. In their supposed cyclic relation to an intermediate host, as reported by Schaudinn, the spirochetes conformed to the accepted observations of the time regarding protozoa rather than bacteria. In their behavior toward chemical reagents on the other hand, the spirochetes stood midway between the more resistant bacteria and the less resistant trypanosomes. The resistance of the bacteria Swellengrebel believed depended not upon a difference in property of protoplasm, but upon the structure of the cell wall. His final conclusions are that, except for their longitudinal division, the spirochetes differ from bacteria

scarcely more than various recognized bacteria differ from each other. In 1907 Swellengrebel had classified the spirochetes with spirilla, but in 1912 he stated that this was hardly permissible since the characteristics which spirochetes have in common with spirilla, except the form are also present in other bacteria. Hence, he finally classified the spirochetes with the bacteria and places them in the family *Spirochaetaceae*, distinct from the *Coccaceae*, *Bacteriaceae* and *Spirillaceae*. This is where Ehrenberg placed them originally.

Dobell also in 1912 (9) reported the results of extensive observations on spirochetes and related organisms from which he concludes that "the spirochetes may be collected into a single group which may be called Spirochaetoidea; they are noncellular (unicellular?) organisms (Protista) and undoubtedly belong to the Schizophyta (Bacteria and Cyanophyceae), and not to the protozoa. Among the Schizophyta they must be placed in the sub-division Bacteria and among the Bacteria they probably constitute a group of the same systematic status as the Cocci, the Bacilli, and the Spirilla." He states that the spirochetes differ from the bacteria in only one feature and that is, that although they are actively motile, they possess no specialized organs of locomotion. At the present time however, as summarized by Ford (19), it is recognized that spirochetes differ from bacteria in internal structure, mode of locomotion, staining reactions, and filterability.

Classification.

The nomenclature of this group of organisms has been in a chaotic state because of the various opinions of the different investigators, and a number of classifications have been proposed. Migula suggested the following in 1897:

Bacteria.....(Coccaceae, Bacteriaceae, Spirillaceae, Chlamydo-
bacteriaceae, and Beggiatoceae)

Spirillaceae

Spirosoma.....Rigid; no organ of locomotion
Microspira.....Rigid; one polar wavy flagellum
Spirillum.....Rigid; polar tufts of flagella, semicircular or
wavy
Spirochaeta.....Flexuous, motion organ unknown, probably
an undulating membrane

In 1912 Gonder classified the spirochetes as follows:

Spirochaeta.....	Type: <i>Spirochaeta plicatilis</i> , etc., all free
Ehrenberg, 1838.....	living
Cristispira.....	Type: <i>Cristispira balbianii</i> , and other varieties
Gross, 1910.....	found in mussels
Spiroonema.....	Type: <i>Spiroonema recurrentis</i> , and other para-
Vuillemin, 1905.....	sitic and pathogenic varieties living in blood
Treponema.....	Type: <i>Treponema pallidum</i> , and other varie-
Schaudinn, 1905.....	ties with closely set spirals

Dobell, in his classification of 1912, had no *Spiroonema*, but classified those organisms with *Treponema*. Gross also did this in 1912. In addition he created a new group known as *Saprospira*, the organisms being similar to *Cristispira* but possessing no crista.

In 1912 Levaditi also classified the spirochetes in the following manner:

Spirillaceae (Migula)

1		2
Sub-family, Spirillaceae		Sub-family, Spirochaetacea. Spirally flex- ible, surrounded by periplastic cover, with or without flagellum-like appendages.
Not flexible		
Genus:	Genus:	Genus: Spirochaeta
Spirillum	Vibrio	<i>S. gallinarum</i>
		<i>S. duttoni</i>
		<i>S. obermeieri</i>
		<i>S. buccalis</i>
		<i>S. balanitidis</i>
		<i>S. refringens</i>
		Genus: Treponema
		<i>T. pallidum</i>
		<i>T. framboesia</i>
		<i>S. dentium</i>

In 1918 Noguchi placed spirochetes in the following classes: (a) *Spirochaeta*, (b) *Cristispira*, (c) *Saprospira*, (d) *Leptospira*, (e) *Spiroonema*, and (f) *Treponema*.

(a) The investigations of Zuelzer (67), Gross (21), Dobell (10), and Swellengrebel (60) as well as others have shown that the organism for which Ehrenberg created the name *Spirochaeta* is distinct from the majority of so-called spirochetes. (b) The genus *Cristispira* was proposed by Gross in 1910 (22) and was characterized by the presence of a membranous structure running from one end of the body of the organism to the other in a spiral fashion and assuming the appearance

of a crista or ridge; the body has a chambered structure and a strong flexible membrane. The type organism was first described by Certes in 1882 and was found in oysters (6). (c) The genus *Saprospira* was also proposed by Gross in 1910 (23) and includes several varieties of spiral organisms which are found in mussels, but differ from *Cristispira* in that they possess no crista. (d) The genus *Leptospira*, proposed by Noguchi, includes those organisms which have minute closely set, regular primary spirals and pointed ends which may be semicircularly hooked. The first organism of this type was described by Wolbach and Binger (64), being isolated from stagnant water and known as *Spirochaeta biflexa* and later as *Leptospira biflexa*. The type organism of this group is (27) *Leptospira icterohemorrhagiae* (Inada, et al.). (e) The genus *Spironema* as proposed by Gonder includes the blood parasites, having *Spironema recurrentis* as the type organism. These organisms have large, wavy, inconstant spirals about five in number, with pointed ends, while the genus *Treponema* (f) with *Treponema pallidum* as the type organism, includes those delicate organisms with regular rigid spirals and pointed ends. They are usually tissue parasites.

There is little difficulty in distinguishing *Spironema*, *Saprospira*, *Cristispira*, and *Leptospira* from each other, but to differentiate between *Treponema* and *Spironema* is not always easily done. The distinction between these two groups depends chiefly upon the rigidity and regularity of the spirals which are characteristic of the *Treponema*. The greater variability and irregularity of the spirals and greater relative diameter are usually seen in *Spironema*. Under natural conditions there is not much difficulty in classification but according to Noguchi (39) the *Spironema* may acquire such a rigidity that it appears as a *Treponema*, especially in cultures. Dobell (10) and Gross (21) regarded the distinction between *Treponema* and *Spironema* insufficient to maintain two separate genera. Dobell selected the name *Treponema*, while Gross chose *Spironema* for the same group of organisms.

In 1918 Noguchi agreed with Gonder in the classification of *Spironema* and *Treponema* and placed them in two separate groups, but in 1928 (41) he followed Dobell and classified them all under *Treponema*.

In this report both the terms *Spironema* and *Treponema* are retained. Because of the marked difference in these two types of organisms, as defined previously, it is hardly felt justifiable to designate both by one term, *Treponema* or *Spironema*. The term "spirillum"

is here used for a form which may be clearly defined. In the early literature the words "spirilla" and "spirochetes" are often used interchangeably and one must bear in mind the accepted classification of the time at which a particular article was written in order to understand the meaning. Even at the present time the term spirillum is used on the one hand to designate spirilla proper and on the other hand any spiral form. In this report the term "spirilla" is used to designate organisms with rounded ends, often with terminal flagella. These organisms appear rigid and are slow in their movement as compared to the spirochetes, larger, coarser, and usually coiled in one or two shallow spirals. Often they appear as a cylindrical screw.

Although there are instances in which the morphology of a spirochete may not be sharply characteristic of either a treponema or spironema, the typical forms as described above are so different that the two terms convey two distinct pictures and their use is of descriptive value. The spirochetes, as distinguished from the spirilla, have pointed ends, more closely coiled spirals from 3 to 30 in number, are smaller (except for the cristispira and saprospira) and have a most remarkable flexibility never observed in the spirilla.

Spirochetes in birds.

In 1890 Sacharoff (47) reported spirochetes in the blood of geese suffering from a fatal malady characterized by fever, wasting, and diarrhoea. The joints of the bird are very tender so that the slightest handling causes cries of pain. According to Sacharoff the disease affects geese and ducks only. Masses of the responsible spirochetes are present in the blood stream. The organism, known as *Spirochaeta anserina*, is between 10 and 20 microns long and multiplies by transverse division.

In 1903 *Spirochaeta gallinarum* was described by Marchoux and Salimbeni (33), having been found in sick hens in Rio de Janeiro. The infected birds are somnolent, have diarrhoea, and refuse nourishment. At the height of the disease large numbers of spirochetes may be found in the blood. At autopsy the liver shows fatty degeneration and the spleen is about three times the normal size. The infection is transmitted by ticks. The spirochetes which are found in the blood are actively motile and measure 10 to 12 microns in length and 0.25 micron in width (Novy). The affliction is known as fowl spirochaetosis and has been observed in India, Cairo, South Australia, South Algeria, Austria, and southern United States.

Borrel, in 1906 (3) expressed the opinion that *Spirochaeta anserina*

and *Spirochaeta gallinarum* are the same, but this is not consistent with Sacharoff's report that ducks and geese only are susceptible to the former.

Spirochaeta granulosa was discovered by Balfour in 1907 in the spirochaetosis of fowls in the Sudan (1). This organism is also found in the blood and a stage in its life cycle is believed to take place inside the erythrocytes. Clinically the disease is similar to the Brazilian fowl spirochaetosis but it shows recurrences which have not been observed in the Brazilian infection.

Spirochaeta neuweuxi was reported by Brumpt in 1909 (5) in fowl spirochaetosis in Senegal. This strain, he states, is less virulent to fowls than *Spirochaeta gallinarum* and is also virulent to geese, ducks, mice, and Javanese sparrows.

In 1910 Fantham (15) found a spirochete in the blood of the grouse, naming it *Spirochaeta lagopodis*. This organism is from 10 to 18 microns in length, has an undulating membrane, and is transmitted from one bird to another by the castor bean tick, *Ixodes ricinus*.

Spirochaeta lovati was also found in 1910 by Fantham in the caecum of the grouse. The organism is from 16 to 32.5 microns long, with pointed ends. It also possesses an undulating membrane and according to his report multiplies by both transverse and longitudinal fission.

Wolf (66) in 1912 described various forms of spirochetes which were found in geese, ducks, chickens, and doves. The organisms were present in the mouths of these birds with the exception of the dove, which had them in the throat. They have no resemblance to the spirochetes in the human mouth, being coarser in form and staining by ordinary methods. The age and living conditions seemed to have no effect on the presence of the organisms.

Cicic in 1926 (7) reported the presence of spirochetes in the mouth stomach, and large intestine of cows, pigs, chickens, and only in pigs does he report them as being present in the caecum.

Material and methods.

In a preliminary survey the blood of several healthy sparrows, owls, and chickens revealed no spirochetes, examinations being made in Michigan and in Maryland. Thereupon a more thorough examination was made of approximately fifty sick chickens, or discards, obtained from the wholesale poultry dealers in Baltimore. These birds had been shipped from Virginia, West Virginia, Pennsylvania, and rural Maryland. Most of them died shortly after arriving in the laboratory but a few made remarkable recoveries when given proper food and care and were later used for experimental purposes.

All the material examined was observed under the dark field, the most satisfactory results being obtained by using thin glass slides and cover slips, the convex mirror, the 4 mm. objective and no. 12.5 eyepiece, and a film of distilled water between the condenser and the bottom of the glass slide. In using the binocular attachment the no. 7.5 eyepieces were most satisfactory. Examinations were also made under oil, using the 1.9 mm. and the 12.5 eyepiece. This was preferable when the material under examination was somewhat sluggish and it was desired to obtain more detail, although there was no special advantage in using it for the determination of structure in spirochetes. In making stained preparations the films were fixed in various ways, by the use of heat, methyl alcohol, and osmic acid. Giemsa and Fontana stains were employed but satisfactory results were not always obtained since a field showing large numbers of spirochetes under the dark field might show only very few when stained. The Kliewe method of spirochete staining was then used (29). By this method the film is prepared as usual, air-dried, and fixed by passing several times through a Bunsen flame. It is mordanted with a 0.5 to 1 per cent aqueous solution of potassium permanganate, washed in water, stained with 1:20 solution of carbolfuchsin and finally washed in water. In staining mouth spirochetes and those of relapsing fever the mordant and stain are allowed to act from 1½ to 3 minutes each. For *Treponema pallidum* however, the mordant should act from 3 to 5 minutes with slight warming and the stain also be left on the slide for 3 to 5 minutes. The method for *Treponema pallidum* as thus employed was found very satisfactory in staining the spirochetes which were under observation. The greater number of spirochetes were stained and there was no difficulty in demonstrating spirochetes in the films if the organisms were fairly numerous under the dark field. However, the small, short, and more delicate treponemas which were present in the raw material did not always stain as well as did the coarser forms, in fact many were only faintly stained if at all. To make photomicrographs of the film stained in this manner was unsuccessful as far as the treponemas were concerned. A greater contrast was desired as well as a stain which would permit the more delicate forms to stand out more clearly. The method was therefore modified as follows: a 2 to 5 per cent aqueous solution of methyl violet was used instead of the carbolfuchsin and the time for the action of the mordant and the stain was increased up to 8 or 10 minutes; the mordant was gently warmed as was done for *Treponema pallidum*. This gave a much sharper contrast on the slide, the organisms stained a deep purple or black and the more delicate

forms which could not be satisfactorily stained by the original method appeared much more distinct. Films stained in this manner were satisfactorily photographed and were also used in the drawings. Crystal violet was also used and proved very satisfactory. Basic fuchsin was tried instead of carbol-fuchsin and seemed to show no difference in staining. Also carbolic acid was added to the methyl violet stain, but no difference in staining was observed over the aqueous solution. The modified Kiewe method of staining spirochetes was used throughout the examinations of all the material. Not only is it simple in preparation and use, but it seems to stain practically all the organisms seen under the dark field, which could not be done by any other method, including the original Kiewe. The modified stain was found to give very satisfactory results also with *Treponema pallidum*, *Spirochaeta Novyi*, *Spirochaeta obermeieri*, *Spirochaeta Duttoni*, *Leptospira icterohemorrhagiae*, and *Treponema Vincenti*, as well as the water leptospira.

In staining the living organisms, Victoria blue stain was employed (phenyl-naphthyl-methane) since the organisms seemed to withstand a higher concentration of this stain than of malachite green, cresyl blue, and crystal violet. Alcoholic solutions of the Victoria blue varying in strength from 0.5 to 1.5 per cent were employed. A perfectly clean slide was flooded with the stain and placed on end to allow the excess to drain off. Within a few minutes the slide was dry and had on it a thin film of the stain. A loopful or drop of the suspension of spirochetes was then placed on the slide, covered, and examined with a 4 mm. objective. The advantage of this method is that a large number of slides may be prepared and kept for future use. The larger spirochetes were stained satisfactorily and remained alive, although their activity was somewhat decreased. Some strains seemed to show more resistance and remained alive for a half hour. The shape of the spirochetes was very distinct and granular-like bodies appeared within the organism. These often had the appearance of changing their position within the spirochete with the movement of the organism. The motion of the organism was considerably decreased when brought in contact with the stain, and the granular appearance may be due to an unfavorable environment. The same granular appearance was noted in sluggish organisms under the dark field. There is undoubtedly a disturbance of the cell contents due to unfavorable conditions and it can hardly be said that distinct bodies within the spirochete are stained.

General results.

Routine blood and feces examinations were made on all the birds and after death the liver, spleen, lungs, kidneys, heart, brain, and bone marrow were examined for spirochetes. In one case of so-called "roup" spirochetes were found in the lungs. Examinations of the throat swabs seldom showed spirochetes, and when present they were coarse S-shaped with two or three loosely coiled spirals and pointed ends. Other examinations continued negative with the exception of the feces. Fresh feces were collected from normal chickens as they were brought to the Baltimore markets. It was found that about 25 per cent of the droppings collected at random contained spirochetes. The material containing spirochetes was yellowish-brown, semi-solid or pasty and had a very strong odor, whereas the feces in which no spirochetes were found varied in color from greenish to dark brown, gray, and black, and had a less offensive odor. Upon examination of the entire intestinal tract it was found that the spirochetes were harbored in the caeca, not only in sick chickens, but also in normal adult birds, both hens and roosters. The yellowish material was found to be caecal material. At various markets where poultry is dressed it was possible to obtain an unlimited quantity of chicken intestines and thus an ample supply of material for study was available. Frequently the material was obtained from chickens which had been dressed immediately after killing and the intestines were still warm when brought to the laboratory. For the most part however, the birds had been chilled before dressing. In general there was very little difference between cold and warm material, except that in the warm material the spirochetes showed greater activity.

In obtaining material from the caecum it was found that by cutting the tubes lengthwise with a pair of scissors, washing out the contents with physiological salt solution and then making scrapings of the walls it was possible to obtain specimens of spirochetes almost free from other organisms. Sections of one caecum were prepared according to the Levaditi silver impregnation method and showed the spirochetes (treponemas) lying in the crypts and also in the epithelial cells of the crypts, (figure 1).

The breed of the chicken appeared to have no influence upon the presence or absence of the organisms, since they were found in White Leghorns, White Rocks, Barred Rocks, Rhode Island Reds, and various mixed breeds. The age of the bird however, seems to have a definite relation to the organisms, since, in very young birds and baby chicks these spirochetes were never found. Older chickens served as a much more satisfactory source of material.

Eight one-day old chicks obtained in April 1929 showed no spirochetes in the caecal material or in the intestinal tract. Examinations were made of all droppings, that from the caecum being distinguished by its yellow color. These birds were kept isolated in the laboratory on a diet of grains, greens, powdered milk, and cod-liver oil, with a quantity of peat moss poultry litter in their pens. Examinations made six months later, November 1st, 1929, failed to show spirochetes, and in one chicken which was killed because of illness the caeca were carefully examined under the dark field, the contents as well as the scrapings from the walls. Large numbers of motile bacilli, spirilla, and small active vibrios were present, but no spirochetes could be demonstrated.

Bacteriological examinations and cultures made of specimens collected from chickens on the market showed the predominance of *Bacterium coli*. The samples of chicken droppings were collected as aseptically as possible, a small amount of the fresh material placed in a sterile test tube, and a suspension made with about 5 or 10 cc. of sterile physiological salt solution. If the suspension appeared heavy a further dilution was made and then a loopful of the material was added to tubes of nutrient agar which had been melted and cooled to 45° C., and then poured into Petri dishes. After a 48-hour period of incubation, colonies were picked, films made and stained by Gram's method. The various types were planted in plain broth, incubated for 24 hours and then transferred to the following media: lactose, dextrose, saccharose, and nitrate broths, Clark-Lubs, Voges-Proskauer, peptone (for indol), litmus milk, potato slants, lead acetate agar slants, gelatin and meat tubes. *Bacterium coli* was isolated from 24 out of 25 specimens. Other organisms isolated were *B. lactici acidii*, *B. subtilis*, *B. cereus*, *B. alcaligines*, *Micrococcus percitreus*, *Micrococcus subflavus*, *B. paratyphosus* (in one specimen), *Achromobacter agile*, and a number of unidentified cocci and bacilli. The bacterial flora of the specimens containing spirochetes showed no difference from that which contained none.

In making examinations of the fresh living material (mounted in Ringer's solution) under the microscope one observes a great activity in the field which is especially striking under the dark field. The bright field was wholly unsatisfactory except if vital stains were used. If the fresh fecal material was under examination the large numbers of other organisms overshadowed the spirochetes, if present. The various motile bacilli had a rapid progressive, gliding movement and hastened across the field or in and out of the debris, the longer ones bending somewhat but showing no elasticity or flexibility of the walls

such as is seen in the spirochetes. The spirilla present in some instances, appeared somewhat awkward and stiff in their movement, and had what seemed to be a single flagellum at one end. The motion was relatively slow and they had the cork-screw-cylinder appearance with $\frac{1}{2}$ to 2 turns, the ends being distinctly blunt or rounded. Cocci and non-motile bacilli merely drifted about in the field. The delicate spirochetes however, showed the greatest activity and when so many other organisms were present they would not always be observed readily, especially by the inexperienced. Their position could be noted under the dark field by what may be referred to as "little flashes of light", and when focussing on these one was able to see the spirochetes. Not much could be determined of their shape or the number of coils until their activity was somewhat decreased. This was especially true of the treponemas with their closely wound spirals. The spironemas had coils which were more loosely wound and were also wider than the treponemas, had about 5 coils, and also distinctly pointed ends. Flexible fusiform bacilli could also be seen which were strikingly different from the other motile bacilli. Various protozoa were occasionally noted,—trichomonadae and amoebae. If caecal scrapings were used for examinations there was less interference of the bacteria and the spirochetes, when present, could be studied satisfactorily.

Types of spirochetes observed.

The difference between the spirilla and spirochetes was definite. However, it was found that the spirochetes which were observed could be placed into three different groups: (1) Treponema, (2) Spironema, and (3) Fusi-spirochaeta types.

Type 1, the treponema, is a very delicate organism, 0.3 to 0.5 micron in diameter with closely wound spirals and pointed ends. The organism is extremely flexible, has a very active progressive cork-screw-like movement, bends without losing its spiral shape, and may be seen burrowing into the debris. The short forms with 3 or 4 spirals and from 5 to 7 microns long are especially common in the fresh fecal material while in the cultures the longer forms appear. These will be described in detail under "Cultivation."

Type 2, the spironema is a loosely coiled organism with pointed ends, 0.5 to 0.75 micron in diameter, having from 3 to 5 spirals and varying in length from 8 to 10 microns. The activity of this organism is not as great as that of the treponemas, often being quite sluggish in specimens in which the delicate treponemas are very active. The organism is flexible and moves about progressively in serpentine fashion in any direction.

Type 3, the fusi-spirochaeta is perhaps the most striking. Certain fusiform bacilli dart back and forth with considerable rapidity within a small area, about 15 microns or less, and then take the form of spirochetes, the number of spirals varying with the length of the organism. The spirochaetal form of this organism resembles the spironema rather than the treponema. Some of the slender fusiforms may often be seen bent on themselves. The fusi-spiral changes from bacillus to spirochete and vice versa occur with great rapidity and also in rapid succession. There is a marked flexibility of the fusiforms showing this change and the action may be described as resembling that of a bent or coiled spring, when stretched out the organism appears as a bacillus. On the other hand some of the fusiforms which show this jerky motion may have the shape of a bacillus on the forward dart and that of a spirochete on the backward dart, or vice versa, since there is no indication of polarity. Also, the organism may move along progressively as a spirochete, stop, and appear as a fusiform bacillus, and then again continue along its progressive path for some distance as a spirochete and stop again. It was thought at first that this difference in appearance might be due to a turning of the organism, but this does not appear to be the case.

Several caeca were kept in Petri dishes at ice-box temperature and dark field examinations were made after 24 hours. Only occasional treponemas were found in each specimen and these appeared granular and motionless, and of irregular shape; the spironema, although present, also appeared motionless and sometimes granular, but the fusiform bacilli of various lengths were present in large numbers and showed slight activity. After a half hour or more at room temperature a marked change appeared on the slide. Activity appeared throughout the specimen and the darting fusiforms were beginning to show a spirochaetal character in form and motion, while the treponemas and spironemas showed no revival even after several hours. The fusi-spirochetes became sluggish after 12 hours on the slide at room temperature and then became inactive. One specimen however, failed to behave in this manner. After two days at ice-box temperature the treponemas and spironemas could be seen. They were inactive, but within a half hour at room temperature had regained some of their activity. The darting fusiforms were not observed in this particular specimen.

Cultivation.

Fresh fecal material containing spirochetes was planted on nutrient agar, tubes, and plates, and also in broth tubes. The bacteria grew

profusely, while the spirochetes disappeared completely within twenty-four hours. This held true even after the plates and tubes were kept for several weeks on the assumption that the bacteria might eventually die out and thus leave the field free for the spirochetes. It appears however, that they were completely destroyed by bacteria and their products.

A suspension of caecal scrapings was made in Ringer's solution. This was filtered through a Mandler filter to 6-7 pounds pressure and upon examination of the filtrate under the dark field it was found that the smaller more delicate forms of spirochetes, i.e. the treponemas, passed through the filter. The first few drops of the filtrate contained only treponemas which were still active though somewhat distorted in appearance. These could be seen under the dark field and also in films stained with the modified Kliewe stain. Small granular bodies also passed through the filter with the treponemas. Material collected later showed in addition bacteria which had also made their way through the filter. The first half cubic centimeter or less was found most suitable for culturing and inoculations were made in various media. Cultures were incubated at 20°, 38°, 42°, and 55° Centigrade, both aerobically and anaerobically. Some tubes were also placed in an atmosphere of ammonia. Anaerobic incubation was done with the modified Varney jar (8), and in some series in test tubes sealed with vaseline (table).

That all filterable micro-organisms are not absolutely beyond the limit of microscopic vision has been known since 1898 when Nocard and Roux (38) cultivated the organism which caused pleural pneumonia in cattle. Novy and Knapp (43) first stated that an infective stage of the spirochetes of relapsing fever may pass through a Berkefeld filter. This was also reported by Breinl and Kinghorn (4), and gave support to the theories advanced by Leishmann (30), Balfour (2), Hindle (26) and Fantam (16) that the chromatin-like granules in the blood spirochetes of man and fowls are a definite stage in reproduction, and when cast off, these granules are capable both of independent reproduction as such and of developing into spirochetes. Wolbach (65) states that this subject is one of great importance because of its bearing upon the classification of the spirochetes. He believed that if granules could be proved to be a stage in the reproduction it would furnish evidence of their protozoal nature. This is not necessarily the case however, since it has now been shown that certain bacteria pass through a granular stage. These observations have been made on the tubercle bacillus by Sweany (56) and Kahn (28) and in dissociating cultures of

B. coli, *B. typhosus*, *B. pneumoniae*, *B. leprosepticum*, *B. malleus*, and *B. diphtheriae* by Hadley (25).

The medium which gave repeated positive results in cultivating the caecal organisms which had passed through the filter was that which was used by Noguchi in the cultivation of *Treponema pallidum* (42). It consisted of 2 parts of nutrient agar, 1 part of sterile ascitic fluid, and a piece of fresh sterile tissue, rabbit or guinea pig liver or kidney, in a test tube covered with a layer of sterile paraffin oil and incubated aerobically at 38° C. for three or four days. On one agar plate early in the investigation, the raw feces were streaked over the surface and the culture placed in the modified Varney jar at 38° C. This particular plate developed a mold beneath which on the agar was a good growth of the larger spirochetes, the spironema, but the treponemas were not observed. In spite of several attempts this could not be repeated.

It was noted in general that the tubes containing fresh liver, either rabbit or guinea pig, gave the most satisfactory results. If these tubes were sealed with vaseline no growth was obtained, but if overlaid with paraffin oil and incubated aerobically at 38° C. a good growth could usually be observed on the third day. Inoculations of the tubes were made by means of Pasteur pipettes, using a drop or so of the filtrate. Macroscopically, there seemed to be no visible change in the tubes in which the spirochetes grew. When examined microscopically however, the organisms were found along or very close to the line of inoculation in cultures several days old, while later examinations showed that they were also growing outwardly toward the edge of the tube. Examinations of the tubes on the first and second days after the inoculation showed no spirochetes or bacteria under the dark field. Very active ovoid bodies were observed in some tubes, some of these bodies appearing slightly elongated. On the third day the treponemas were usually present in large numbers and transverse division was actively taking place. The maximum growth seemed to be reached about the fourth day. The longer treponemas were extremely active and in watching such an organism it was soon seen that near the center there began to appear a "thinning," that is, the spirals seemed to stretch out giving the organism the appearance of consisting of two halves connected by means of a fine thread. Then there followed bending, twisting, and pulling motions as if trying to tear apart until separation finally took place. There is another striking behavior in this process of division. When the two halves of the organism bend toward each other (incurvation), they may be suddenly wrapped around each other, appearing as a single spirochete, except that the ends remain free, resembling a

forked tail. Seeing this form thus, without having observed just what happened might cause one erroneously to think that longitudinal division was taking place. These two spiral halves may uncoil again and continue various movements until finally separated. Clumps of treponemas are quite common, especially in tubes showing a heavy growth (fig. 3). On the sixth and seventh days the tubes show the darting fusiform bacilli and fusi-spirochetes in addition to the treponemas, and in tubes several months old granular forms are observed which are striking. The organism may have three or four coils, be very much enlarged, and consist of granular bodies about the size of the active coccoid bodies previously noted. When stained, these granular spirochetes appear as streptococci, the units being distinct. These granular forms in old cultures were also seen to disintegrate. Cultures seven months old, with no transfers made, showed not only the darting fusiforms but also treponemas still growing. These tubes were kept at 37° C., the pH of the media being 6.9. The pH of fresh caecal material containing spirochetes was 6.8.

Earlier workers in spirochetes frequently reported longitudinal division in this group of organisms. Prowazek and most of the German school of protozoologists considered that spirochetes divide longitudinally, while Novy, Swellengrebel, Laveran, Noguchi, and others believe that the division is only transverse. Fantham (17) reported very interesting results in connection with observations on the division of *Spironema duttoni* and *Spironema recurrentis*, and also found the Y-stage which he considered longitudinal division. Mackinnon (32) also observed the Y-stage and concluded that it was longitudinal division. More recent workers however, are of the opinion that spirochetes divide only transversely, and that what was formerly considered longitudinal division, and truly appeared as such, was merely a stage in the transverse division.

Observations made of cultures kept in a constant temperature chamber at 37° C. remained alive for several days, showed transverse division and darting fusi-spiral forms, and then became sluggish.

Fresh caecal scrapings containing the three types of spirochetes were subjected to the action of distilled water, 10 per cent saponin solution, and 10 per cent oxgall. When mounted in distilled water instead of Ringer's solution the spirionemas lost their activity and appeared as fusiform bacilli. This loss of activity was not a general quiet slowing down, but the organism gave intermittent jerks, and appeared to have a struggle, until action ceased completely after about ten minutes. The treponemas did not lose their shape, but activity

disappeared within a minute and they drifted about the field. The fusi-spirochetes seemed to be unaffected by the distilled water. With 10 per cent saponin the treponemas and spironemas were completely dissolved in less than 30 seconds. Some fusi-spirochetes remained but soon they also lost their activity. The bacteria present seemed to be unaffected. The oxgall solution also dissolved the spirochetes.

Various culture media used.

Media	Aerobic	Anaerobic	Ammonia	Temp.
Raw material on agar + mold	—	+	—	38° C.
Broth, agar, sterile chicken kidney	—	—	—	38° C. 42° C.
Broth, agar, sterile kidney (chicken)	—	—	—	"
Semi-solid agar + rabbit blood	—	—	—	" 45° C.
				" 55° C.
Semi-solid agar, sterile feces	—	—	—	" 45° C.
				" 55° C.
Rabbit blood	—	—	—	"
Chicken serum	—	—	—	" 55° C.
Caecal extract and agar	—	—	—	"
Coagulated egg yolk	—	—	—	"
Semi-solid agar, chicken blood	—	—	—	"
Caecal extract agar, and ammonium acetate	—	—		"
Caecal extract agar, ammonium tartrate and carbonate	—	—		"
Plates of agar, gelatin, ammonium carbonate, piece of caecum	—	—	—	38° C.
Ascitic fluid	—	—	—	42° C.
N. N. N.	—	—	—	"
Agar plates and trypsin	—	—	—	"
Agar and mold	—	—	—	"
Gelatin and mold	—	—	—	25° C.
Winogradsky glucose flask	—	—	—	38° C.
Winogradsky mannite flask	—	—	—	"
Serum and agar plate	—	—	—	" 55° C.
Blood clot on agar	—	—	—	"
Mold and serum on agar	—	—	—	"
Ascitic fluid, agar, sterile tissue, and paraffin oil	+	—	—	" 42° C.
Ascitic fluid and agar	—	—		(—)
Meat tubes	—	—		"

Method of obtaining spirochetes by surgical operation.

Since the spirochetes were found to be localized in the caeca, only material obtained from this source was suitable for the study of these organisms. However, the caeca appeared to be evacuated only at irregular intervals so that one might wait several days before spirochaetal material could be obtained. To have a ready source of spirochetes available, caecostomy was performed under aseptic precautions. The chicken was anesthetized with ether, strapped on its back to the surgical board, the field of operation plucked and disinfected with cresol and covered with a sterile operating cloth. After preliminary exploration it was found that the blind end of a caecum could be most easily reached through an incision about 2-3 cm. lateral and parallel to the midline, just anterior to the anus. With the retraction of the body wall and the viscera the blind end of one of the caeca was grasped with a hemostat and pulled through the incision. The peritoneum and the body wall were closed in layers, leaving an adequate sized open tract for the caecum. This was sutured to the deeper layer and to the skin by four stitches at each level of fixation, allowing about 1 cm. of the caecum to protrude beyond the skin. Black silk sutures were used throughout. A dressing of dry sterile gauze was applied and secured with a many-tailed bandage crossed over the back and body. After four days the protruding end of the caecum had dried off and was removed. The caecum could be entered with a slightly curved, slender, blunt-end glass tube, about 4.5 cm. long. In the aspiration of caecal contents a syringe was connected to the glass tube with rubber tubing. Physiological salt solution was injected when the caecal contents were too hard for easy aspiration. Microscopical examination of material thus obtained showed the desired spirochetes. In one young chicken the caecum yielded no spirochetes, but the old chickens that were operated all gave good material. The birds seem to be unaffected by this procedure, making an uneventful recovery in every case. When entering the caecum later at intervals to obtain spirochetes it was not necessary to anesthetize the bird since there seemed to be no pain or discomfort. One bird kept for five months after the operation was re-examined. The wound had accidentally closed but the tip of the caecum was still adherent to the inner surface of the body wall. The caecum was again opened from the exterior and satisfactory material obtained.

Pathogenicity.

Suspensions of caecal material containing spirochetes (in Ringer's solution) were filtered through a Mandler filter and doses of 1 cc. were

fed daily for a week to chicks which were several days old. Preliminary examinations of all droppings for several days were negative for spirochetes. Intramuscular injections of 1 cc. were given to three chicks less than a week old. Seven days later one of the chicks which had received an intramuscular injection died rather suddenly. Complete examination of blood, tissue, bone marrow, and intestinal tract showed no spirochetes, nor were there any gross pathological changes,—the liver, lung, and heart appeared normal, but the spleen seemed very much enlarged. One of the chicks which had received seven oral doses also died and in this case spirochetes were found in the intestinal tract and a few were demonstrated in the kidneys.

The filtrate containing spirochetes, was also injected intraperitoneally into white rats in doses of 1.0, 2.0, and 3.0 cc. Temperatures were taken daily for a week and no change was observed. The animals appeared unaffected by the injections.

One cubic centimeter of the concentrated suspension of the culture of treponemas and fusi-spirochetes was injected into the testis of a young rabbit. There appeared to be no effect during the three weeks of observation. The same amount of material was also injected subcutaneously into a guinea pig with no resulting reaction.

Fantham (18) described the act of penetration of epithelial cells in the human feces by spirochetes, an observation which he believed suggested that they may similarly enter the cells which line the intestinal canal. It was suggested that spirochetes are at any rate a potential danger, and may be capable of pathogenic activity either by spreading into the blood vessels and producing a generalized infection, or by an invasion of the cells of the intestinal walls, or even by mechanical injury.

Synopsis of characteristics of spirochetes observed.

Type 1, the spirochete of the treponema type, resembling *Treponema pallidum* is found in the caecum of the adult chicken. It varies in length from 5 to 7 microns in the fresh material, up to about 12 microns in the cultures. The width is from 0.3 to 0.5 micron. It is colorless, has closely wound spirals from 5 to 12 in number, depending upon the length of the organism. Measurements made of the organism in living material agreed with those of films stained by the modified Klieve method. The measurements made on fuchsin stained material, however, were somewhat larger than those of the living organism, especially the width. The organism appears to be devoid of flagella and nothing could be determined of the internal structure since the

spirochetes appeared uniformly stained throughout the specimens. Motility consists of rapid cork-screw motion in which the organism moves forward and backward, and of bending about without losing the spiral form. The ends of the organism are pointed and no anterior-posterior polarity has been observed. In culture "incurvation" is common and transverse division into two is the means of multiplication. Longitudinal division was never observed in either the fresh material or the cultures. The spirochetes obtained from the culture tubes and examined under the dark field are commonly seen to penetrate the agar medium, and burrow their way through it. While in the agar the organisms are stretched out and appear irregularly waved and bent in various directions, seemingly following courses of less resistance. If too difficult an obstacle is encountered the organism may be seen to attempt to get through, or penetrate, and then give up as it seems; back away from the obstacle and move around it. When an organism which has thus worked its way through agar or tissue or detritus again emerges into a free or open space it at once appears uniformly and closely coiled. The living organisms cannot be seen under the bright field unless vital stains are used and then only their general form may be observed, it being impossible to ascertain anything of the internal structure because of the small size and delicate character of these organisms. This type of organism passes readily through a Mandler filter and grows under semi-anaerobic conditions in a medium consisting of two parts of nutrient agar, one part of sterile ascitic fluid, and sterile rabbit or guinea pig liver, and incubated at 37° C. for three or four days. A layer of sterile paraffin oil was placed over the medium after inoculation. Levaditi sections of the caecum show that the organisms penetrate the epithelium. This type of organism appears to be non-pathogenic, since chickens, rabbits, guinea pigs, and rats were unaffected by various inoculations. It has survived in a culture tube for more than seven months, still showing active dividing forms after that time. Tentatively this organism is designated as *Treponema caeci-gallorum*, nov. sp. (fig. 4).

Type 2, the spironema type, is an organism which measures 0.5 to 0.75 micron in diameter and varies in length from 8 to 10 microns, with 3 to 5 spirals which are loosely coiled. The organism has pointed ends and there are no indications of flagella. Motility consists of rapid serpentine-like movements. Nothing could be determined of the internal structure when stained in films, but with vital stains irregular granules were observed. This irregular granular structure is also evident in the organisms which have lost their motility and may be

observed under the dark field. In Levaditi sections this type was observed in the crypts but showed no penetration. The organism is not filterable and hence it was impossible to obtain it free from bacteria. Attempts at cultivation were unsuccessful it is believed because of the presence of the bacteria. The spirochete is non-pathogenic so far as can be ascertained. It is designated tentatively as *Spironema caeci-gallorum*, nov. sp. (fig. 2).

Type 3, the fusi-spiral type, is an organism varying in length from 5 to 15 microns and from 1 to 2 microns wide at the widest point, or across the center of the organism. The ends are distinctly tapering and pointed. Various degrees of motility may be observed in one field. Often the fusiforms bend on themselves, form a circle or loop, while others show a marked flexibility of a different character and appear as spirals or undulations of 3 to 5 waves, with pointed ends, and extremely motile. There is active backward and forward movement which is progressive also. Those appearing as spirochetes seem to have rigid spirals and do not show the bending and lashing movements in addition to the cork-screw-like motion. In stained films the various stages may also be observed,—some are fusiforms, others show several curves or shallow spirals, while still others show definite uniform curves all with pointed ends. It is possible that these forms pass through the filter. Although not observed as either fusiforms or spirochetes in the filtrate, they nevertheless appeared in the treponema culture tubes. Nothing has been definitely determined, but there are two possibilities, either they pass through the filter as granules, or they constitute a phase in the life cycle of the spirochetes (fig. 5). This organism is designated tentatively as *Fusi-spirochaeta caeci-gallorum*, nov. sp.

Discussion.

Chickens serve as a very convenient source of spirochetes, especially suitable for morphological observations. Some of the strains are readily cultivated and can be handled with safety because of their non-pathogenic character. Although the spirochetes may be transferred to baby chicks by feeding, there is no satisfactory evidence that the death of the one chick was due to spirochetes. The question arises, when and where do chickens acquire spirochetes? Judging from the habits of the bird it would not be at all difficult to assume that they obtain these organisms by the ingestion of food and water which has been contaminated with feces from older birds carrying spirochetes. Once established in the caeca the organisms seem to remain there throughout the life of the chicken. Whether they play a secondary

role in infections has not been determined. Mayer (34) reports spirochetes and fusiform bacilli in the intestines of normal cats. The animals were experimentally infected with amoebae, sections made of the lesions, no amoebae could be demonstrated, but spirochetes and fusiform bacilli appeared to have penetrated. The fusiforms went as far as the submucosa, while the spirochetes went into the submucosa and even into the muscular layer.

The filtrate which was used as inoculum showed not only the active treponemas, but also granular bodies. In cultures of the treponemas there later appeared the fusi-spiral forms together with the treponemas. On some days a tube which had shown a heavy growth of the treponemas, *Treponema caeci-gallorum*, might show huge numbers of active fusi-spirochetes while only few treponemas were present. Just how the fusi-spiral type gets into the culture is not known, it was never seen in the filtrate, either as a spirochete or as a fusiform bacillus. It may be that it granulates and the granules pass through the filter. If this does not occur, the question arises if the fusi-spirochetes are merely a stage in the development of the treponemas.

The relation between fusiform bacilli and spirochetes has been given considerable attention, especially in case of Vincent's angina, and the literature contains many reports. Rukawischnikoff (46) studied the biology of the fusiforms and the spirochetes and concludes that they are different stages on the same organism. Sanarelli (49) observed that the spirochetes of Vincent grew especially well in the presence of *B. mesentericus* and after several days changed to fusiform bacilli. This transformation he reports is labile and it was possible to again obtain the original spirochaetal forms by sub-culturing in peptone water. If the spirochetes were exposed for any length of time to the air they always changed to fusiform bacilli. He further states that fusiform bacilli are nothing more than spirochetes which through some external influence have lost their ability to carry on their screw-like rotation on their own axis. He also made extensive studies on the caecal spirochetes in the rabbit and guinea pig and observed the fusi-spiral forms in his cultures (50). In 1905 Mackie (31), while discussing Vincent's angina and the fusiform bacillus, made the statement that he believed that the spiral organism, the fusiform bacillus, and sickle-shaped body which were described "will all turn out to be different stages in the life cycle of the one parasite." He also states that the fusiform bacillus is not a true bacillus. Wechselmann and Loewenthal observed the presence of fusiform bacilli in spirochaetal material (63) and state that different stages of development or generations may occur

among the spirochetes as was shown by Mayer and Schreyer in relation between fusiform bacilli and spirochetes in Vincent's angina, stomatitis, etc. (35). Aside from the true spirochetes there are other forms which they place in two distinct groups: the straight rod forms and the bent "sausage forms." The rod forms are thin and often pointed at the ends, being $2\frac{1}{2}$ to 3 microns long and $\frac{1}{4}$ micron in thickness. In staining these forms with Giemsa they are said to appear pale blue and show from 1 to 4 reddish granules of chromatic substance which appear to multiply by division. The bent or "sausage" forms are reported as having about the same length but appear somewhat thicker and are more intensely stained by Giemsa. In the center of this type of organism they observed a chromatin, mass which divides into 2 or 4 bodies, two of which are smaller than the other two. These observations of Loewenthal and Wechselsmann were made not only on *Spirochaeta pallidum*, but also on *Spirochaeta refringens*, in which the rod forms had distinctly pointed ends, and also on mouth spirochetes. The rod forms and "sausage forms" found in stomatitis ulcerosa were also studied by these workers. These organisms are very active, the movements being "snake-like" in one plane. In the small forms only half of the body seems to move whereby the body is pushed forward. The smaller forms seemed to have a quick motion in which the entire body was bent and stretched. The rods varied considerably in length, from $2\frac{3}{4}$ to 8 microns, and the ends were for the most part pointed. The small forms showed a chromatin body which divided into eight smaller bodies. In this octo-granular stage the transition into spirochetes was observed. These rod forms also multiplied by division and these workers believe that this is one of the ways of multiplication of spirochetes, aside from transverse division. In regard to the spirochetes in stomatitis they state that the following has been established: the spirochetes which do not have a single nucleus in this form, but have chromatin material distributed in fine dust-like particles, "fall" or break up into short individuals; through shortening and thickening mononuclear rods are formed and these then develop into spirochetes.

Many questions might be raised concerning this work. These workers had considerable difficulty in mastering the technique, but with practice it was possible to obtain spirochetes readily. Material was obtained chiefly from primary lesions, stained films were studied, and fresh living material was observed under the ultramicroscope.

Krystalowicz and Siedlecki in 1908 (30) made studies on the morphology of *Spirochaeta pallidum* and observed various forms in the cultures and state that rod forms as well as spirochetes of various

diameters appeared at different intervals. The presence of bodies resembling cocci in spirochete cultures has been reported by Prowazek (45), Gross (24), Tunnicliff (61), and Muhlen and Hartman (37).

Conclusions.

1. Normal adult chickens in Maryland and vicinity carry spirochetes which are harbored in the caeca and appear to be non-pathogenic.

2. Three distinct types were observed and designated tentatively as: 1, *Treponema caeci-gallorum*; 2, *Spironema caeci-gallorum*; and 3, *Fusi-spirochaeta caeci-gallorum*.

a. *Treponema caeci-gallorum* is a small delicate organism, from 5 to 7 microns long, and in cultures up to 12 microns, 0.3 to 0.5 micron in width, and from 5 to 12 spirals. It is extremely motile. It passes through a Mandler filter as a spirochete and can be cultivated on a medium containing agar, ascitic fluid, and sterile rabbit or guinea pig liver in test tubes overlaid with sterile paraffin oil.

b. *Spironema caeci-gallorum* is an active organism from 8 to 10 microns long, 0.5 to 0.75 micron in width, with pointed ends, and from 3 to 7 loosely coiled spirals. The organism is not filterable and could not be successfully cultivated.

c. *Fusi-spirochaeta caeci-gallorum* is a strikingly distinct organism. It is a fusiform bacillus of various lengths, from 5 to 15 microns, and from 1 to 2 microns wide at the center, which may take on a spirochaetal motion. The organism was observed in the raw material, not in the filtrate, but appeared and persisted in the cultures with the treponemas.

3. Cultures from raw unfiltered material never yielded a satisfactory growth of spirochetes.

4. Levaditi sections of the caecum showed a penetration of the epithelial lining of the crypts by *Treponema caeci-gallorum*, while *Spironema caeci-gallorum* and *Fusi-spirochaeta caeci-gallorum* were present in the lumen of the crypts.

5. A modification of the Kliewe spirochete stain was used throughout in staining films and found most satisfactory.

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PLATE I.

FIG. 1. Photomicrograph of section of caecum showing the penetration of the spirochetes into the epithelial lining of a crypt. Prepared according to the Levaditi method.

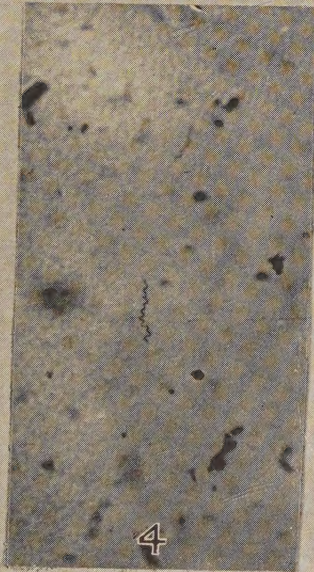
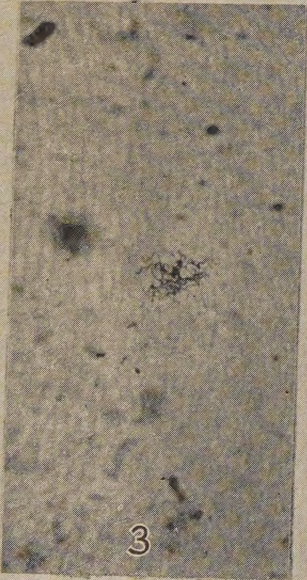
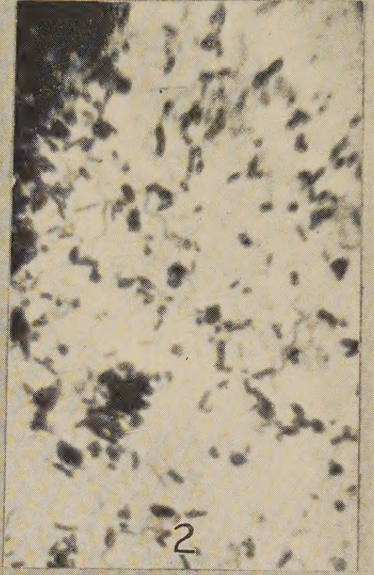
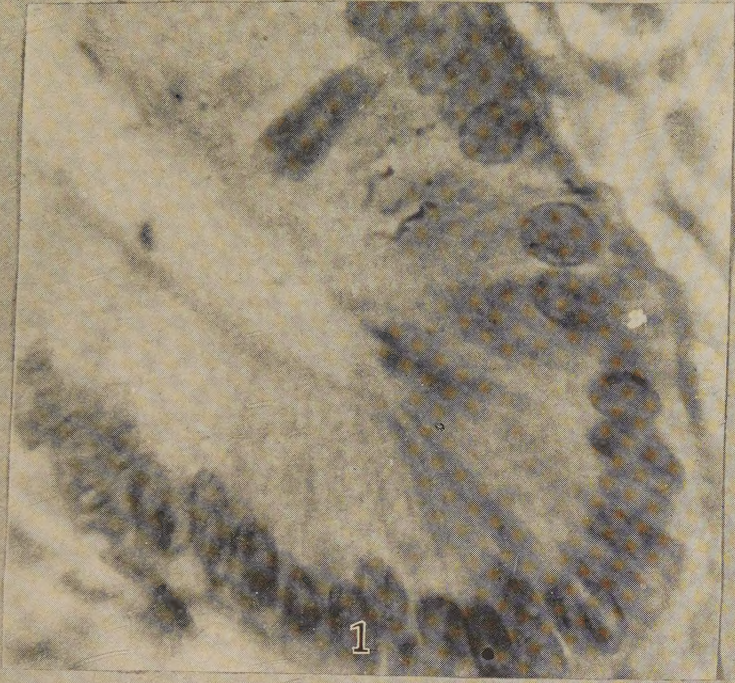
FIG. 2. Film of raw material showing larger spirochetes of the spironema type. Photomicrograph. Stained by Kliewe method.

FIG. 3. Photomicrograph of culture of treponemas showing clumping. Stained by modified Kliewe method.

FIG. 4. Photomicrograph of culture of treponemas. Stained by modified Kliewe method.

FIG. 5. Camera lucida drawing of culture of treponemas showing the fusi-spirochetes and fusiform bacilli. Stained by modified Kliewe method.

PLATE I



V I T A

Minnie B. K. Harris was born in Mt. Clemens, Michigan, April 26, 1895. She graduated from the Mt. Clemens High School, 1913, and received her academic training in the University of Michigan, 1913-1914, 1919-1920; the College of the City of Detroit, 1924-1926; Columbia University, Summer, 1925. She entered the Johns Hopkins University in October, 1926, as a candidate for the degree of Doctor of Philosophy, with Bacteriology as the principal subject. She was appointed Instructor in Botany 2 (Bacteriology) in the Johns Hopkins University, 1930.

